Due to the Notice, certain obvious typographical or clerical errors in the specification and Figure 6 have recently come to light. The Examiner's consideration and entry of the present amendment correcting the inadvertent errors is respectfully requested. The proposed correction to Figure 6 is highlighted in red on the attached copy of the drawing. The proposed corrections to pages 9 (lines 23-25), 31 (Table 1) and 42 (Example 10) are found below in the Appendix. The full, corrected pages are included herein as substitute sheets, if deemed acceptable. No new matter is introduced into the application by the amendment.

As the Examiner will appreciate from the following explanation, there is a reasonable basis for making the changes and the errors would be quite obvious to one of ordinary skill in the art. Support for the corrections is found in the disclosure and the full-length sequence of the avian HEV (SEQ ID NO:1), as originally filed.

With respect to Figure 6, the correct 3' non-coding region (NCR) starts with TGT. The 3 nucleotides ACA, which were inadvertently placed in the original Figure 6 and the computer count of 130 nucleotides, are not a part of the 3' NCR and do not exist as such in the full-length sequence. It appears that when Applicants were copying and pasting the 3' NCR sequence into a Word program from the original sequence using MacVector software, the three letters ACA came from another line of sequence. The mistake did not become apparent until Applicants' file version of the 3' NCR was recently compared to the copy recited in the application. However, if a comparison were made between Applicants' full-length sequence of avian HEV containing 3' NCR as described in the patent application and the 3' NCR of Figure 6 as originally filed, the mistake would be noticed. Plus, one of ordinary skill in the art would be able to extract the correct NCR sequence from SEQ ID NO:1.

Regarding the specification, it became clear upon review of Table 1 (highlighted by the Examiner) that most of the positions of the partial sequences relative to the full-length SEQ ID NO:1 were inaccurate and needed correction. During this process, it also came

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to light that the only difference between Applicants' avian HEV sequence of 3931 base pairs and Figures 9A-9C is the 15 nucleotides contained in the Poly(A) tail at the 3' end (see also SEQ ID NO:1). Because the 5' sense primer is not present in Figures 9A-9C, the mention of the primer sequence as a part of the drawing on page 9, lines 23-25, of the specification is an obvious, innocent mistake that should be corrected.

Upon further inspection of Table 1, some of the sequences could not be found in the full-length sequence of SEQ ID NO:1. Two sequences were exact duplicates yet the positions were listed in both descending and ascending orders. Two other sequences were placed in the table in descending positions, yet they were actually located in ascending order in SEQ ID NO:1. Looking at the way the position numbers were written, it became clear that the primers in ascending position were meant to be forward primers, the primers in descending order were intended to be reverse primers, and many of those reverse primers were accidentally written as sense sequences when antisense primers were intended and used in the description.

It is well-known in the art that a reverse primer is the complementary sequence to the forward one. Since primers are written 5' to 3', it is standard practice to write reverse primers as both reverse and complementary to keep the role of 5' to 3'. Moreover, reverse primers have to be made reverse and complementary for PCR.

In text, Applicants state that both strands of the approximately 4 kb fragment were sequenced using primer walking strategy (see Example 4 on page 32 and Claim 1 drawn to the complementary strand). The specification describes the use of forward and reverse primers to determine the nucleotide sequence of the 4 kb viral genome. It could not be done by PCR unless Applicants had used both forward and reverse primers. Although Applicants had written the positions in descending order to make the point clear that those partial sequences were reverse primers, they accidentally wrote several reverse primers as sense sequences. Unfortunately, the error was not noticed before now.

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Nevertheless, the mistakes in Table 1 are merely typographical or clerical errors that would be obvious to one of ordinary skill in this art. Since the primers are totally derived from the full-length sequence as originally described and filed, a person of ordinary skill in the art would be able to determine the correct positions or nucleotide sequences for all of the sense and antisense primers. As such, these corrections should not be considered new matter.

To meet the requirements of 37 C.F.R. § 1.821-1.825, a substitute paper copy of the Sequence Listing and a computer readable form (CRF) copy on computer disc are enclosed. As mandated, the present amendment directs the entry of the Sequence Listing into the application. The Sequence Listing is incorporated by reference into the application on page 1 of the specification. It is noted that all of the reverse primers from the corrected Table 1 have been included in the Sequence Listing for completeness.

Pursuant to 37 C.F.R. § 1.821(f), it is hereby stated that the Sequence Listing information recorded in computer readable form is identical to the content of the written paper copy of the Sequence Listing. It is further stated in accordance with 37 C.F.R. § 1.821(g) that this submission does not include new matter.

On a final note, the undersigned attorney would like to thank the Examiner for her guidance in the telephone call of August 19, 2002 with regard to correcting the sequence in the drawing. The errors in Table 1 became apparent afterwards but it is believed that the same guidelines would hold true for correcting those sequences. If the Examiner has any questions relating to the present amendment or would like a declaration by the inventor, she is invited to contact the undersigned.

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Accordingly, the record with respect to the formalities is now complete and the application is ready for examination. Favorable treatment is urged.

Respectfully submitted,

VIRGINIA TECH INTELLECTUAL PROPERTIES, INC.

Date: October 3, 2002 By: Anne M Rosenblum

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FILING BY EXPRESS MAIL UNDER 37 C.F.R. § 1.10

This correspondence is being deposited with the U.S. Postal Service on October 3, 2002 to be delivered by the "Express Mail Post Office to Addressee" service under Mailing Label Number EK461082857US addressed to: Box SEQUENCE, Commissioner for Patents, Washington, D.C. 20231.

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APPENDIX

MARKED UP VERSION OF PERTINENT SECTION OF SPECIFICATION

Page 1, lines 18-21

REFERENCE TO A "Sequence Listing"

The material on a single compact disc containing a Sequence Listing file provided in this application is incorporated by reference. The date of creation is [March 22] September 9, 2002 and the size is [14] approximately 21.4 KB.

Page 8, lines 18 - 29

Figures 2A and 2B represent the amino acid sequence alignment of the putative RNA-dependent RNA polymerase (RdRp) gene of avian HEV (which corresponds to SEQ ID NO:4) with that of known HEV strains. The conserved GDD motif is underlined. The sequence of the prototype Burmese strain is shown on top, and only differences are indicated. Deletions are indicated by hyphens (-).

Figures 3A-3C represent the sequence alignment of the ORFs 1, 2 and 3 overlapping region. The sequence of the prototype Burmese strain is shown on top, and only differences are indicated in other HEV strains. The sequence of avian HEV (which corresponds to SEQ ID NO:12) is shown at the bottom. The start codons are indicated by arrows, and the stop codons are indicated by three asterisks (***). The two PCR primers (FdelAHEV and RdelAHEV) used to amplify the region flanking the deletions are indicated. Deletions are indicated by hyphens (-).

Page 9, lines 3 - 13

Figures 5A-5C represent the amino acid sequence alignment of the putative capsid gene (ORF2) of avian HEV (which corresponds to SEQ ID NO:6) with that of known HEV strains. The putative signal peptide sequence is highlighted, and the predicted cleavage site is indicated by arrowheads. The N-linked glycosylation sites are underlined in boldface. The sequence of the prototype Burmese strain is shown on top, and only differences are indicated in other HEV strains. The conserved tetrapeptide APLT is indicated (asterisks). Deletions are indicated by hyphens (-).

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Figure 6 illustrates the sequence alignments of the 3' noncoding region (NCR) of avian HEV (which corresponds to SEQ ID NO:13) with that of known HEV strains. The 3' NCR of avian HEV is shown on top, and only differences are indicated in other HEV strains. Deletions are indicated by hyphens (-).

Page 9, lines 23-25

Figures 9A-9C represent the entire 4 kb nucleotide sequence (3931 bp plus poly(a) tract at 3' end [and 5' sense primer for amplification]) of the avian hepatitis E virus (which corresponds to SEQ ID NO:1).

Page 31, Table 1

Sequencing Primers and Positions:

[TTGGTGGGGTGCTGGTCGAGATTG	438-415]	
CAATCTCGACCAGCACCCCACCAA (SEQ ID NO:14)	407-384	
CCGGGAGCGCTGTAGTGTGATTGATGT	[389-414]	358-384
[CCATAAATCCACCCGGGCCTGT	649-628]	
ACAGGCCCGGGTGGATTTATGG (SEQ ID NO:15)	618-597	
CAATCAACCCCTCAACACTGGA	[871-892]	840-861
[ATTTACGCTGGATGACCCTGTTGCAC	1038-1013]	
GTGCAACAGGGTCATCCAGCGTAAAT (SEQ ID NO:16)	1007-982	
GGATGCCCGATTGGATGGTAGCCTT	[1306-1330]	1275-1299
[GGATGCCCGATTGGATGGTAGCCTT]		
AAGGCTACCATCCAATCGGGCATCC(SEQ ID NO:17)	[1330-1306]	1299-1275
TCCCGGGAGCTGGTGTTGTCTGC	[1633-1655]	1602-1624
GATGCCCGATTGGATGGTAGCCTTGTA	[1307-1333]	1276-1302
[CTGACAAGAACTGGGGGCCCGACAT	1708-1644]	
ATGTCGGGCCCCCAGTTCTTGTCAG (SEQ ID NO:18)	1677-1653	
CAATGTGCTGCGGGGTGTCAAG	[2046-2067]	2015-2036
[AATGTGCTGCGGGGTGTCAAGGG	2069-2047]	

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CCCTTGACACCCCGCAGCACATT (SEQ ID NO:19)	2038-2016	
[CAAATGCGGTGGGCGGCTTCTCTATA	2470-2445]	
TATAGAGAAGCCGCCCACCGCATTTG (SEQ ID NO:20)	2439-2414	
[ACTGCTGAGGATGGCGAAATTGGTC	2945-2921]	
GACCAATTTCGCCATCCTCAGCAGT (SEQ ID NO:21)	2914-2890	
ACCGACATATACAGTTTCACCTCAG (SEQ ID NO:22)	[3072-3096]	3065-3041
CTGAGGTGAAACTGTATATGTCGGT	[3096-3072]	3041-3065
GAACGGCGAGCCTGAGGTGAAACTGT	[3061-3086]	3030-3055
[TTCTCTATAAGCATGGCCTATTG	2486-2462]	
CAATAGGCCATGCTTATAGAGAA (SEQ ID NO:23)	2453-2431	
[CAGAATGGTAGCTCCGTGGTTTGGTATGC	3603-3575]	
GCATACCAAACCACGGAGCTACCATTCTG (SEQ ID NO:24)	3572-3544	
TCTTCAGAATGGTAGCTCCGTGGTTTG	[3571-3597]	3540-3566
GCCGCGGTGACAACGTCTGTGAGAGG (SEQ ID NO:25)	[2143-2168]	2168-2143

[a] The positions are relative to the 3931 bp sequence of avian HEV (corresponding to SEQ ID NO:1) determined in the present invention.

Page 36, lines 1 - 8

were selected and sequenced for both DNA strands. The number of poly (A) residues at the 3' end of each of the three cDNA clones was different (19, 23, and 26 residues, respectively), indicating that these 3 clones sequenced represent independent cDNA clones. This 4 kb genomic fragment contains the complete ORFs 2 and 3 (set forth in SEQ ID NO:7 and SEQ ID NO:9, respectively), the complete RNA-dependent RNA polymerase (RdRp) gene (set forth in SEQ ID NO:5), a partial helicase gene of the ORF1 (set forth in SEQ ID NO:3), and the complete 3' noncoding region (NCR) (set forth in SEQ ID NO:13).

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Paragraph bridging pages 36 and 37

The incomplete ORF1 sequence of avian HEV was aligned with the corresponding regions of human and swine HEV strains. Significant nucleotide and amino acid sequence identities were found in the ORF1 region between avian HEV and known HEV strains (Table 2, below). The avian HEV ORF1 region sequenced thus far contained the complete RdRp gene and a partial helicase gene. The RdRp gene of avian HEV encodes 483 amino acid residues and terminates at the stop codon of ORF1. A GDD motif (positions 343-345 in RdRp gene) that is believed to be critical for viral replication was identified (Figs. 2A-2B corresponding to SEQ ID NO:4), and this motif was found in all RdRps (G. Kamer *et al.*, "Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses," Nucleic Acids Res. **12**:7269-7282 (1984)). The RdRp gene of avian HEV is 4 amino acid residues shorter than that of known HEV strains (Figs. 2A-2B corresponding to SEQ ID NO:4), and shared 47% to 50% amino acid and 52% to 53% nucleotide sequence identity with that of known HEV strains (Table 2, below). The C-terminal 146 amino acid residues of the incomplete helicase gene of avian HEV shared approximately 57-60% nucleotide sequence and 58-60% amino acid sequence identities with the corresponding region of other HEV strains. The helicase gene of avian HEV is the most conserved region compared to known HEV strains. There is no deletion or insertion in this partial helicase gene region between avian HEV and other HEV strains. A 439 bp sequence of BLSV is available in the helicase gene region (C. J. Payne *et al.*, 1999, *supra*), and avian HEV shared 80% nucleotide sequence identity with BLSV in this region.

Pages 39 and 40

EXAMPLE 9

Sequence Analysis of the ORFs 2 and 3

The ORF2 gene of avian HEV consists of 1,821 nucleotides with a coding capacity of 606 amino acids, about 60 amino acids shorter than that of other HEV strains. The ORF2 gene of avian HEV overlaps with ORF3 (Figs. 3A-3C corresponding to SEQ ID NO:12), and terminates at stop codon UAA located 130 bases upstream the poly (A) tract. The predicted amino acid sequence of ORF2 contains a typical signal peptide at its N-terminus followed by a hydrophilic domain (Fig. 4). The sequence of the avian HEV signal peptide is distinct from that of known

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HEV strains (Figs. 5A-5C corresponding to SEQ ID NO:6). However, it contains common signal peptide features that are necessary for the translocation of the peptide into endoplasmic reticulum: a positively charged amino acid (Arginine) at its N-terminus, a core of highly hydrophobic region (rich in Leucine residues) and a cleavage site (SRG-SQ) between position 19 and 20 (Figs. 5A-5C corresponding to SEQ ID NO:6). Sequence analysis of the ORF2 revealed that the region between the signal peptide and the conserved tetrapeptide APLT (positions 108-111) is hypervariable, and 54 amino acid residues of avian HEV are deleted in this region (Figs. 5A-5C corresponding to SEQ ID NO:6). Three putative N-linked glycosylation sites were identified in the ORF2 of avian HEV: NLS (pos. 255-257), NST (pos. 510-512) and NGS (pos. 522-524). Three N-linked glycosylation sites were also identified in known HEV strains but the locations are different from those of avian HEV. The first glycosylation site in known HEV strains is absent in avian HEV (Figs. 5A-5C corresponding to SEQ ID NO:6), and the third glycosylation site in avian HEV is absent in the known HEV strains.

The ORF2 gene of known HEV strains varies slightly in size, ranging from 655 to 672 amino acid residues, but most strains have a ORF2 gene of 660 amino acid residues. The ORF2 of avian HEV has 606 amino acid residues, which is 54 amino acids shorter than that of most known HEV strains. The deletions are largely due to the shift of the ORF2 start codon of avian HEV to 80 nucleotides downstream from that of known HEV strains (Figs. 3A-3C corresponding to SEQ ID NO:12). The putative capsid gene (ORF2) of avian HEV shared only 42% to 44% amino acid sequence identity with that of known HEV strains (Table 3, below), when the major deletion at the N-terminus is taken into consideration. However, when the N-terminal deletion is not included in the comparison, avian HEV shared 48% to 49% amino acid sequence identity with the corresponding region of other HEV strains.

Multiple sequence alignment revealed that the normal start codon of the ORF3 gene in known HEV strains does not exist in avian HEV due to base substitutions (Figs. 3A-3C corresponding to SEQ ID NO:12). Avian HEV utilizes the ORF2 start codon of other HEV strains for its ORF3, and consequently the ORF3 of avian HEV starts 41 nucleotides downstream from the start codon of known HEV strains (Figs. 3A-3C corresponding to SEQ ID NO:12). Unlike known HEV strains, the ORF3 gene of avian HEV does not overlap with the ORF1 and locates 33 bases downstream from the ORF1 stop codon (Figs. 3A-3C corresponding to SEQ ID

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NO:12). The ORF3 of avian HEV consists of 264 nucleotides with a coding capacity of 87 amino acid residues, which is 24 to 37 amino acid residues shorter than that of known HEV strains. Sequence analysis indicated that the ORF3 of avian HEV is very divergent compared to that of known HEV strains.

Page 42, line 1 to Page 43, line 5 (now line 6)

EXAMPLE 10

Sequence Analysis of the 3' NCRs

The region between the stop codon of the ORF2 and the poly (A) tail of avian HEV, the 3' NCR, is [130] 127 nucleotides (set forth in SEQ ID NO:13). Sequence analysis revealed that the 3' NCR of avian HEV is the longest among all known HEV strains. The 3 NCRs of known HEV strains range from 65 to 74 nucleotides (Fig. 6 corresponding to SEQ ID NO:13). Multiple sequence alignment indicated that the 3' NCRs of HEV is highly variable, although a stretch of sequence immediately proceeding the poly (A) tract is relatively conserved (Fig. 6 corresponding to SEQ ID NO:13).

EXAMPLE 11

Identification of a Major Deletion in the ORFs 2 and 3

Overlapping Region of Avian HEV

Sequence analyses revealed a major deletion of 54 amino acid residues in avian HEV between the putative signal peptide and the conserved tetrapeptide APLT of the ORF2 (Figs. 5A-5C corresponding to SEQ ID NO:6). To rule out the possibility of RT-PCR artifacts, a pair of avian HEV-specific primers flanking the deleted region was designed (Table 1, Figs. 3A-3C). The 3' antisense primer (RdelAHEV) located before the ORF3 stop codon of avian HEV, and the 5' sense primer (FdelAHEV) located within the C-terminal region of the ORF1. To minimize potential secondary structure problems, reverse transcription was performed at 60°C with a One Step RT-PCR Kit (Qiagen Inc., Valencia, CA). PCR was performed with 35 cycles of denaturation at 95°C for 40 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute. In addition, PCR was also performed with shorter annealing time and higher denaturation temperature to avoid potential problems due to secondary structures. The PCR

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reaction consisted of an initial enzyme activation step at 95°C for 13 minutes, followed by 35 cycles of denaturation at 98°C for 20 seconds, annealing at 55°C for 5 seconds and extension at 73°C for 1 minute. It has been reported that formamide or DMSO could enhance the capability of PCR to amplify certain genomic regions of HEV (S. Yin *et al.*, "A new Chinese isolate of hepatitis E virus: comparison with strains recovered from different geographical regions," Virus Genes **9**:23-32 (1994)). Therefore, a sufficient amount to make 5% (v/v) of formamide or DMSO was added in the PCR reactions. A PCR product of the same size (502 bp) as observed in a conventional PCR is produced with various different RT-PCR parameters and conditions including the addition of 5% (v/v) of formamide or DMSO, the use of higher denaturation temperature and short annealing time, and the synthesis of cDNA at 60°C (Fig. 7). The deletion was further confirmed by directly sequencing the 502 bp PCR product.

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